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*****  
*      WELCOME TO THE      *  
*      U. S. PATENT TEXT FILE      *  
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=> s shigella

L1 2076 SHIGELLA

=> s mammal?

L2 48077 MAMMAL?

=> s l1(p)l2

L3 116 L1(P)L2

=> s entry or entered or enter

163758 ENTRY  
97439 ENTERED  
233513 ENTER

L4 385306 ENTRY OR ENTERED OR ENTER

=> s l4(p)l3

L5 3 L4(P)L3

=> d

1. 5,824,538, Oct. 20, 1998, Shigella vector for delivering DNA to a mammalian cell; Arthur A. Branstrom, et al., 435/252.1; 424/93.2; 435/245, 252.3, 455, 822 [IMAGE AVAILABLE]

=> d 1 parn

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L5: 1 of 3

=> d fd parn

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L5: 1 of 3  
DATE FILED: Sep. 6, 1995

=> d xa xp

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L5: 1 of 3  
ASST-EXMR: Christopher R. Tate  
PRIM-EXMR: Leon B. Lankford, Jr.

=> d clms

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L5: 1 of 3

CLAIMS:

CLMS(1)

What is claimed is:

1. A delivery vehicle for delivering a mammalian expression plasmid into a mammalian cell, said vehicle comprising a pure culture of attenuated Shigella cells into which said expression plasmid has been introduced, wherein said Shigella cells have at least one genetic mutation such that they lyse once inside said mammalian cell, thereby delivering said expression plasmid therein.

CLMS(2)

2. The delivery vehicle according to claim 1, wherein said Shigella is *S. flexneri*.

CLMS(3)

3. The delivery vehicle according to claim 1, wherein said mammalian cell is a cell of an intestinal mucosal epithelium.

CLMS(4)

4. The delivery vehicle according to claim 3, wherein said Shigella is S. flexneri.

CLMS(5)

5. The delivery vehicle according to claim 4, wherein said S. flexneri is strain 15D given ATCC accession number 55710.

CLMS(6)

6. The delivery vehicle according to claim 1, wherein said attenuated Shigella cells are inactivated.

CLMS(7)

7. The delivery vehicle according to claim 6, wherein said attenuated Shigella is heat-inactivated.

CLMS(8)

8. The delivery vehicle of claim 1, wherein said mutation is a mutation in the wild-type *asd* gene within said Shigella cells.

CLMS(9)

9. A method for delivering a mammalian expression plasmid into a mammalian cell, said method comprising:  
(i) introducing a mammalian expression plasmid into a pure culture of attenuated Shigella cells, wherein said Shigella cells have at least one genetic mutation such that they lyse once inside said mammalian cell; and  
(ii) administering said Shigella cells to said mammalian cell, thereby delivering said expression plasmid therein.

CLMS(10)

10. The method according to claim 9, wherein said Shigella is S. flexneri.

CLMS(11)

11. The method according to claim 10, wherein said S. flexneri is strain 15D, given ATCC accession number 55710.

CLMS(12)

12. The method according to claim 11, wherein said mammalian cell is a cell of a mucosal epithelium.

CLMS(13)

13. The method according to claim 9, wherein said mammalian cell is a cell of a mucosal epithelium.

CLMS(14)

14. The method according to claim 13, wherein said mucosal epithelium is intestinal mucosal epithelium.

CLMS(15)

15. The method according to claim 9, wherein said attenuated Shigella cells are inactivated.

CLMS(16)

16. The method according to claim 15, wherein said attenuated Shigella is heat-inactivated.

CLMS(17)

17. The method of claim 9, wherein said mutation is a mutation in the wild-type asd gene within said Shigella cells.

=> d his

(FILE USPAT ENTERED AT 16:19:52 ON 13 JAN 1999)  
L1 2076 S SHIGELLA  
L2 48077 S MAMMAL?  
L3 116 S L1(P)L2  
L4 385306 S ENTRY OR ENTERED OR ENTER  
L5 3 S L4(P)L3

=> s aspartate(4a)dehydrogenase

3866 ASPARTATE  
6789 DEHYDROGENASE  
L6 138 ASPARTATE(4A)DEHYDROGENASE

=> s l1 and l6

L7 9 L1 AND L6

=> d 1-4

1. 5,855,880, Jan. 5, 1999, Avirulent microbes and uses therefor; Roy Curtiss, III, et al., 424/93.2, 93.48, 184.1, 200.1, 235.1, 257.1, 258.1; 435/252.3, 252.33, 320.1, 879 [IMAGE AVAILABLE]

2. 5,855,879, Jan. 5, 1999, Avirulent microbes and uses therefor; Roy Curtiss III, 424/93.2, 93.48, 184.1, 200.1, 235.1, 257.1, 258.1; 435/252.3, 252.33, 320.1, 879 [IMAGE AVAILABLE]

3. 5,840,483, Nov. 24, 1998, Method of maintaining a desired recombinant gene in a genetic population of cells; Roy Curtiss, III, 435/6, 252.3, 252.33, 320.1 [IMAGE AVAILABLE]

4. 5,824,538, Oct. 20, 1998, \*\*Shigella\*\* vector for delivering DNA to a mammalian cell; Arthur A. Branstrom, et al., 435/252.1; 424/93.2; 435/245, 252.3, 455, 822 [IMAGE AVAILABLE]

=> d 4 fro

US PAT NO: 5,824,538 [IMAGE AVAILABLE] L7: 4 of 9  
DATE ISSUED: Oct. 20, 1998

TITLE: \*\*Shigella\*\* vector for delivering DNA to a mammalian cell  
INVENTOR: Arthur A. Branstrom, Rockville, MD  
Donata R. Sizemore, Gaithersburg, MD  
Jerald C. Sadoff, Washington, DC

ASSIGNEE: The United States of America as represented by the  
Secretary of the Army, Washington, DC (U.S. govt.)

APPL-NO: 08/523,855  
DATE FILED: Sep. 6, 1995  
INT-CL: [6] C12N 1/00; C12N 1/20; C12N 15/00

US-CL-ISSUED: 435/252.1; 424/93.2; 435/172.1, 172.3, 245, 252.3, 822  
 US-CL-CURRENT: 435/252.1; 424/93.2; 435/245, 252.3, 455, 822  
 SEARCH-FLD: 424/234.1, 235.1, 93.2; 435/245, 172.3, 252.1, 252.3, 822,  
 172.1

## REF-CITED:

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5,077,044	12/1991	Stocker	424/235.1
5,672,345	9/1997	Curtiss, III	424/93.2

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- Galan et al. *Gene*, vol. 94, pp. 29-35, 1990.
- Hatten et al. *Gene*, vol. 129, pp. 123-128, 1993.
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ART-UNIT: 161

PRIM-EXMR: Leon B. Lankford, Jr.

ASST-EXMR: Christopher R. Tate

LEGAL-REP: Charles H. Harris, John Francis Moran

## ABSTRACT:

We describe a bacterial delivery system for the delivery of DNA and antigens into cells. We constructed an attenuated bacterial vector which enters mammalian cells and ruptures delivering functional plasmid DNA, such as a mammalian expression plasmid, and antigens into the cell cytoplasm. This \*\**Shigella*\*\* vector was designed to deliver DNA to colonic surfaces, thus opening the possibility of oral and other mucosal DNA immunization and gene therapy strategies. The attenuated \*\**Shigella*\*\* is also useful as a vaccine for reducing disease symptoms caused by \*\**Shigella*\*\*.

17 Claims, 11 Drawing Figures

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L2 48077 S MAMMAL?  
L3 116 S L1(P)L2  
L4 385306 S ENTRY OR ENTERED OR ENTER  
L5 3 S L4(P)L3  
L6 138 S ASPARTATE(4A)DEHYDROGENASE  
L7 9 S L1 AND L6

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